

BRIEF NOTE

Characterization of Hydrophobic Stream Bacteria Based on Adhesion to n-Octane¹

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ABSTRACT. The purpose of this study was to characterize stream bacterial communities based on cell surface hydrophobicity. Because hydrophobicity is related to adhesion we hypothesized that more hydrophobic bacteria would be found on solid surfaces than in water. Water, rock, and sediment from two northeastern Ohio streams were sampled and bacteria were plated on modified nutrient agar. Hydrophobicity was determined by measuring adherence to n-octane. No difference was found in the proportion of hydrophobic bacteria among habitats. Two hydrophobic isolates were identified as *Sphingomonas paucimobilis* and *Chryseomonas luteola*. A large proportion of hydrophobic bacteria were gram positive and urease positive; none were gelatinase positive. More hydrophobic than hydrophilic bacteria were able to grow using manatose or malatose as the only carbon source. These physiological differences indicate that hydrophobic bacteria may be able to utilize resources not available to hydrophilic bacteria.

OHIO J. SCI. 97 (3): 59-61, 1997

INTRODUCTION

Bacteria are an important component of stream biofilms (i.e., communities of organisms living on solid surfaces) as well as the water column, yet little is known about the characteristics of organisms that comprise biofilm communities. Surface properties of bacteria in biofilms are particularly important and one readily measurable and important characteristic is whether the cell surface is hydrophilic or hydrophobic (Rosenberg and Kjelleberg 1986).

Cell surface hydrophobicity affects nutrient utilization and adhesion and therefore may influence distribution and abundance of bacteria. Hydrophobic bacteria are able to utilize hydrophobic phosphorus substrates more rapidly than hydrophilic bacteria (Lemke et al. 1995). This is an important ability in aquatic environments which are often phosphorus limited (Wetzel 1983) and may release some bacteria from competition for nutrients by allowing them to exploit alternative nutrient sources. Furthermore, hydrophobicity increases adhesion of bacterial cells to surfaces (Daffonchio et al. 1995, Huysman and Verstraete 1993, Stenstrom 1989, van Loosdrecht et al. 1987) and becomes an important factor in streams where erosional forces dislodge bacteria from surfaces (Leff et al. 1994).

We hypothesized that hydrophobic bacteria would have greater adhesive properties and therefore would be found in higher proportion on solid surfaces than in the water. In this study, bacteria from two northeastern Ohio streams were sampled and characterized based on hydrophobic or hydrophilic cell surface properties.

METHODS

Three replicate samples were collected from water, rock, and sediments in Bixon Creek and the West Branch

of the Mahoning River (Portage County, OH) during September, 1995. Water samples were plated on modified nutrient agar, a complex media proven to culture a wide range of bacteria (Leff and Meyer 1991). For rock or sediment samples, 10 g (wet weight) of sample was added to 10 ml sterile stream water, sonicated for five minutes and plated. Plates were incubated at 24° C for three days and colony forming units (CFU) were enumerated. Bacterial isolates (10 per sample) were transferred to nutrient broth and grown to >0.2 optical density (wavelength 600 nm, Spectronic 1001, Bausch and Lomb).

Hydrophobicity of isolates was determined by measuring adherence of suspended bacteria to a hydrophobic solution, n-octane (Rosenberg et al. 1980). Cells were concentrated by centrifugation (14,000 rpm, 30 s, Microcentrifuge 5415C, Eppendorf, Westbury, NY), resuspended in phosphate buffered saline (PBS: 7.6 g NaCl, 1.9 g Na₂HPO₄·7H₂O, 0.7 g NaH₂PO₄·2H₂O per liter, pH = 7.2), which is a hydrophilic solution, and the OD measured at 600 nm (OD₁). The bacterial suspension was mixed for 30 s with n-octane (Sigma Chemical, St. Louis, MO) and allowed to stand for five minutes to insure that the two solutions had separated into the biphasic state. The OD of the bottom hydrophilic layer was measured again (OD₂). Hydrophobicity was calculated using the formula: % adherence = $([OD_1 - OD_2] / OD_1) \times 100$. Isolates with 50% to 100% adherence to n-octane were designated as hydrophobic.

Isolates collected (9 hydrophobic; 18 hydrophilic) were gram stained and gram negative isolates were identified using API test strips for nonfermenting bacteria (API-NFT, bioMerieux Vitek, Inc., Hazelwood, MO). Results were then analyzed to determine if hydrophobic and hydrophilic bacteria differed in response to the various physiological tests in the API-NFT system.

The number of colony forming units were compared by *t*-test and the proportions of hydrophobic bacteria were arcsin transformed and compared by ANOVA (α =

¹Manuscript received 8 August 1996 and in revised form 3 February 1997 (#BN96-19).

0.05, Statview 4.1, Abacus Concepts, Inc., Berkeley, CA).

RESULTS

A similar number of CFU were cultured from each habitat in this study (Fig. 1A). Because CFU were expressed as number per unit volume for water and number per unit mass for rock and sediment they could not be compared. The number of CFU from water samples was not statistically different between streams ($p = 0.79$). The number of CFU also did not differ between rock and sediment ($p = 0.76$). Of the 180 isolates inoculated to nutrient broth, 108 grew to ≥ 0.2 OD₆₀₀ and were tested for hydrophobicity. There was no statistically significant difference in the proportion of bacteria that were hydrophobic among habitats (Fig. 1B, $p = 0.16$).

Almost half (44%) of the hydrophobic bacteria and only 17% of the hydrophilic bacteria were gram-positive. API-NFT strips identified two of the five gram negative hydrophobic isolates as *Sphingomonas paucimobilis*, with a rating of acceptable, and *Chryseomonas luteola*, with a rating of excellent. Seven gram-negative hydro-

philic isolates were identifiable: *Agrobacterium radiobacter*, *Aeromonas hydrophila caviae*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Xanthomonas* sp. and two *Pseudomonas* sp. Individual tests from the API-NFT strips were analyzed to determine if bacteria with different surface characteristics had different physiological properties. Differences were seen in both enzyme and carbon assimilation tests. No hydrophobic bacteria showed gelatinase activity while 67% of hydrophilic bacteria did. The percentage of hydrophobic bacteria that showed urease (20%) and B-galactosidase (60%) activity was three times higher than in hydrophilic bacteria (7% and 20% respectively). Both types of bacteria had similar oxidase responses (40% hydrophobic and 47% hydrophilic). Twice as many hydrophobic isolates grew using malatose (80%) or manatose (80%) as the sole carbon substrate than did hydrophilic bacteria (40% and 47% respectively).

DISCUSSION

Because hydrophobicity increases adhesion (van Loosdrecht et al. 1987), one would expect to find a greater proportion of hydrophobic bacteria attached to surfaces. However, in this study, similar proportions of hydrophobic bacteria were found in all habitats (Fig. 1B). There are two possible explanations for the disagreement between our hypothesis and our results. First, hydrophobic bacteria in the water may be cells that have been released from biofilms or have been washed into the stream from surrounding soil. The majority of aquatic bacteria are gram-negative while most soil bacteria are gram-positive (Rheinheimer 1985). We found 44% of hydrophobic bacteria to be gram-positive. Inputs of allochthonous bacteria into stream water has been demonstrated experimentally (Wainright et al. 1992) and in the field (Edwards et al. 1990; Leff et al. 1993).

Second, hydrophobic bacteria found in the water might be planktonic bacteria that have become hydrophobic to increase their probability of attachment to a surface. Hydrophobicity of a bacterial species may change under different environmental conditions. Although Nikovskaya and Gordienko (1989) found that hydrophobicity was a stable characteristic, independent of growth phase and culturing effects, Allison et al. (1990) concluded that hydrophobicity was a function of growth rate in planktonic bacteria.

Hydrophobic strains differed from hydrophilic isolates in terms of enzyme activity and ability to assimilate different carbon substrates. Results of the enzyme tests reveal differences in function between these bacteria. Urease, which was active in several hydrophobic strains, is required for utilization of organic nitrogen and thus hydrophobic bacteria may exploit nutrients not available to hydrophilic bacteria. Given that the surface of hydrophobic bacteria contains proteins for adhesion that are not found in hydrophilic bacteria (Paul and Jeffrey 1985), proteases (such as gelatinase) that might degrade these proteins would be counterproductive and were absent in hydrophobic strains. Differential utilization of carbon substrates indicates that hydrophobic bacteria may be able to assimilate a wide range of organic

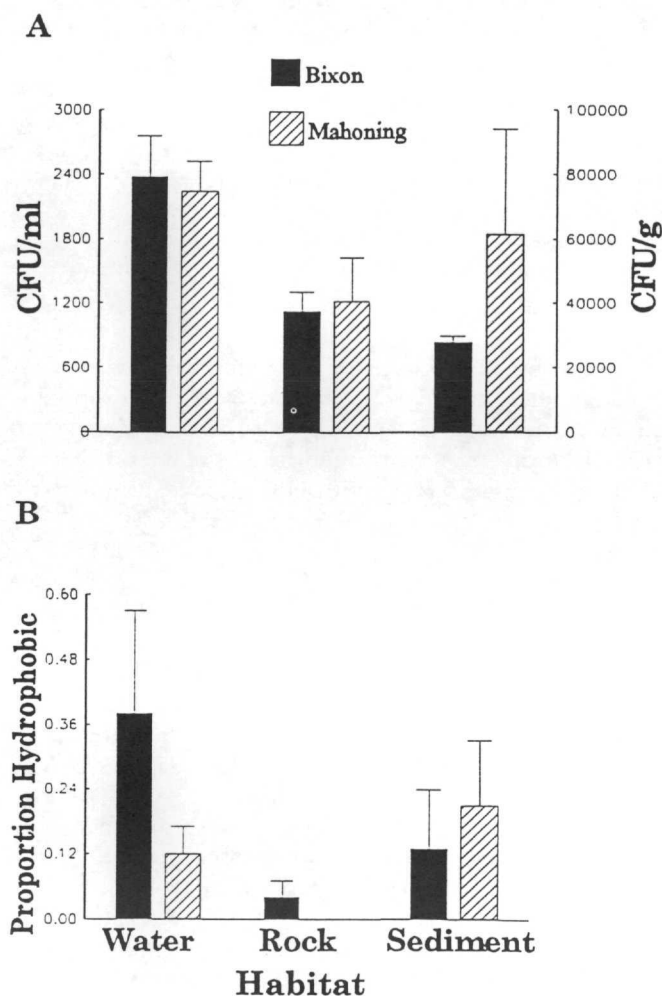


FIGURE 1. A) Number of CFU/ml for water samples and CFU/g wet weight for rock and sediment samples (Mean + SE, $n = 3$). B) Proportion of isolates tested for hydrophobicity with 50-100% adherence to *n*-octane (Mean + SE, $n = 3$). Proportion hydrophobic calculated by dividing the number of hydrophobic isolates by the number of isolates tested for hydrophobicity.

compounds, exploiting energy and carbon sources not available to hydrophilic bacteria.

ACKNOWLEDGEMENTS. Support for this work was provided by Kent State University, Department of Biological Sciences. We thank Conrad E. Wickstrom for his suggestions.

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